Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 1484–1488, February 1991
Genetics

Gene for non-insulin-dependent diabetes mellitus (maturity-onset diabetes of the young subtype) is linked to DNA polymorphism on human chromosome 20q

(DNA marker/adenosine deaminase gene/polymerase chain reaction)

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Communicated by Roger H. Unger, November 28, 1990

ABSTRACT **Maturity-onset diabetes of the young (MODY) is a form of non-insulin-dependent diabetes mellitus characterized by an early age of onset, usually before 25 years of age, and an autosomal dominant mode of inheritance. The largest and best-studied MODY pedigree is the RW family. The majority of the diabetic subjects in this pedigree has a reduced and delayed insulin-secretory response to glucose, and it has been proposed that this abnormal response is the manifestation of the basic genetic defect that leads to diabetes. Using DNA from members of the RW family, we tested more than 75 DNA markers for linkage with MODY. A DNA polymorphism in the adenosine deaminase gene (*ADA*) on the long arm of chromosome 20 was found to cosegregate with MODY. The maximum logarithm of odds (lod score) for linkage between MODY and *ADA* was 5.25 at a recombination fraction of 0.00. These results indicate that the odds are >178,000:1 that the gene responsible for MODY in this family is tightly linked to the *ADA* gene on chromosome 20q.**

Non-insulin-dependent or type 2 diabetes mellitus (NIDDM) is a common disorder of glucose homeostasis affecting ≈5% of the general population. The causes of the fasting hyperglycemia and/or glucose intolerance associated with this form of diabetes are not well understood. The contribution of heredity to the development of NIDDM has been recognized for many years (1), and the high degree of concordance of NIDDM in monozygotic twin pairs (2) indicates that genetic factors play an important role in its development. Since an understanding of the molecular basis of NIDDM would elucidate the mechanisms controlling glucose homeostasis and facilitate the development of more rational therapeutic strategies, we have undertaken a linkage study of NIDDM to identify diabetes-susceptibility genes. The use of linkage strategies to identify DNA markers for NIDDM has been difficult because this disorder does not exhibit simple Mendelian recessive or dominant inheritance. In addition, because of its late age of onset, it is difficult to obtain large multigenerational families suitable for genetic studies. NIDDM is also likely to be genetically heterogeneous, with mutations in several different genes able to cause hyperglycemia, and this heterogeneity could confound the linkage analysis.

There is a form of NIDDM, termed maturity-onset diabetes of the young (MODY; no. 125850 in ref. 3), that has a clear mode of inheritance and for which multigenerational pedigrees have been described (4–6). MODY has been defined as "NIDDM in the young and autosomal dominant inheritance" (5, 6). Although MODY is usually asymptomatic in younger patients, most cases can be diagnosed before the patient is 25 years old, and the disease may be recognized in early adolescence by routine plasma glucose testing. MODY has been identified in many different racial and ethnic groups. However, its prevalence among NIDDM patients in different populations varies considerably and estimates range from 0.15% to 18.5% (5, 6).

The best-characterized MODY pedigree is the RW family (5, 6), which has been studied prospectively since 1958. A great majority of the diabetic subjects in the RW pedigree have a reduced and delayed insulin secretory response to glucose and it has been proposed that the unknown genetic defect is the cause of this abnormal response (5, 6). A similar secretory response to glucose has also been observed in many patients with late age of onset forms of NIDDM (in the absence of islet-cell antibodies). Other MODY families have a "hyperinsulinemic" secretory response to glucose (7) as is seen in the majority of NIDDM patients early in the natural history of their disease. Thus, studies of families with MODY may lead to a better understanding of the etiology of NIDDM. Here we report the mapping of the gene responsible for glucose intolerance in the one MODY pedigree, the RW family, to the long arm of chromosome 20 on the basis of its cosegregation with a DNA polymorphism in the adenosine deaminase gene (*ADA*).

MATERIALS AND METHODS

Subjects. The RW pedigree has been described (5, 6). This family includes five generations and >275 individuals, of whom >185 have been tested for diabetes (Fig. 1). Forty individuals in this pedigree have NIDDM and the age of diagnosis was ≤25 yr for 20 of these patients. Seventy-nine percent of the subjects with NIDDM have fasting hyperglycemia and 32% are insulin requiring. Two individuals in the RW family have insulin-dependent diabetes mellitus (IDDM) and seven have impaired glucose tolerance, a condition that may indicate a tendency to develop NIDDM in the future. In the RW pedigree, the expression and natural history of MODY is variable (6, 7). Among the offspring of II-5 with NIDDM, the fasting plasma glucose levels varied from 91 to 366 mg/dl, and the fasting hyperglycemia developed over an interval ranging from 1.5 years in individual 1247A to 27 years in individual 8106. In some subjects, the fasting hyperglycemia can be controlled with diet alone (individual 8106), whereas in others sulfonylureas (individuals 1246, 1243, 1956,

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Abbreviations: NIDDM, non-insulin-dependent diabetes mellitus; MODY, maturity-onset diabetes of the young; IDDM, insulin-dependent diabetes mellitus; AluVpA, *Alu* variable poly(A).

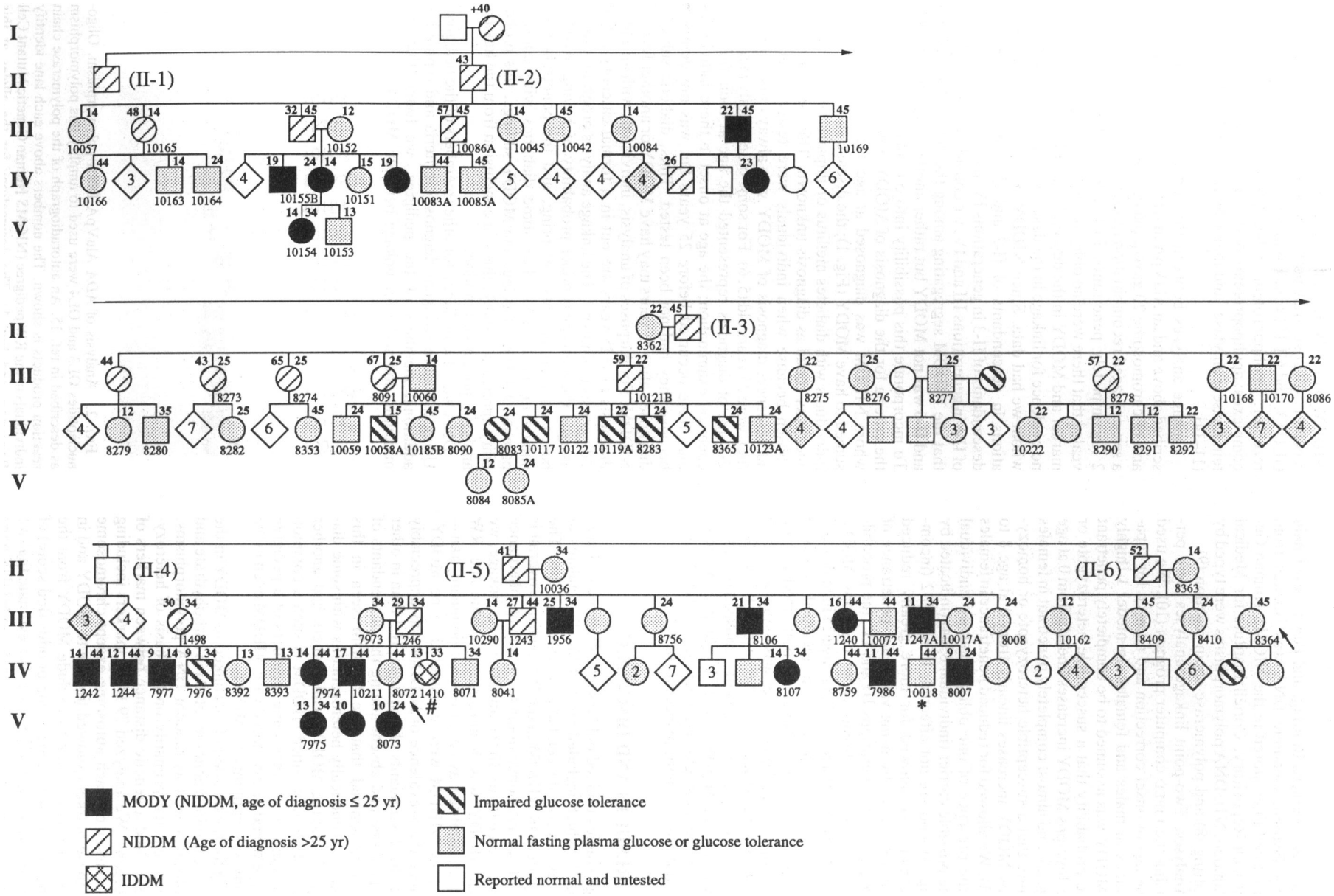

FIG. 1. Partial pedigree of the RW family. The affection status is indicated. The numbers under some subjects are the GM repository numbers assigned by the Coriell Institute for Medical Research (Camden, NJ) to immortalized cell lines stored in the NIGMS Human Genetic Mutant Cell Repository. The age of diagnosis of diabetes mellitus, if known, is indicated at the top left and the *ADA Alu* variable poly(A) (AluVpA) genotype is noted at the top right of the symbol. The full pedigree has been reported (5, 6). The shading represents the "stringent" diagnostic criteria for MODY described in the text. Only NIDDM diagnosed at age ≤25 years is considered to be MODY. NIDDM diagnosed after age 25 may be, but is not assumed to be, MODY.

and 1240) or insulin (individuals 1498 and 1247A) are required (5, 6).

DNA Extraction, Southern Blotting, and Polymerase Chain Reaction Amplification. Genomic DNA was isolated from lymphoblastoid cell lines stored in the NIGMS Human Genetic Mutant Cell Repository, Coriell Institute for Medical Research (Camden, NJ). DNA polymorphisms were typed by Southern blotting (8) and polymerase chain reaction (9).

Linkage Analyses. Two-point linkage analyses were performed by using the LIPED computer program (10). We used a straight-line age of onset correction and allowed for separate penetrances in males and females. In model 1 (highly penetrant), MODY was assumed to be completely penetrant in males (the probability that a susceptible heterozygote or homozygote develops MODY increases linearly from 0 at age 8 to 1.0 at age 25) and almost completely penetrant in females (the probability that a susceptible heterozygote or homozygote develops MODY increases linearly from 0 at age 8 to 0.95 at age 25). We allowed for reduced penetrance in females because of the presence of one obligate carrier (individual 8072) and one possible carrier (individual 8364) (indicated by arrows in Fig. 1) who are not affected. In model 2 (incompletely penetrant), we allowed for more greatly reduced penetrance in both sexes by using values for penetrance of MODY estimated from generations III and IV; the probability that a heterozygote or homozygote develops MODY increases linearly from 0 at age 8 to 0.8 at age 25 in males and to 0.6 at age 25 in females. Model 2 was included to reduce the risk of missing a true linkage. In both models, the penetrance for the normal homozygote was assumed to be 0 throughout life and the frequency of the susceptibility allele for MODY was assumed to be 0.0001, consistent with a lifetime prevalence for MODY of 0.02%. The phenotype of individuals with impaired glucose tolerance or IDDM was considered "unknown" (with respect to MODY).

RESULTS AND DISCUSSION

Initial linkage studies utilized only a segment of the pedigree shown in Fig. 1, which included most of the descendants of II-5 as well as several descendants of II-3 and II-6. The segregation pattern of NIDDM in the RW pedigree overall is consistent with an autosomal dominant mode of inheritance with high penetrance (5, 6). In the initial tests for linkage, we assumed that MODY was homogeneous within the RW pedigree and transmitted as an autosomal dominant trait—i.e., all cases of NIDDM were considered to be MODY. However, since the prevalence of NIDDM was appreciably lower among the descendants of individual II-3 than in other parts of the pedigree and absent among the descendants of II-6, we also recognized that the NIDDM seen in this pedigree might be genetically heterogeneous with some diabetic individuals having MODY while others had another form of NIDDM. In fact, since NIDDM is such a common disorder, it is quite likely that some members of a pedigree that is this large would be diagnosed with NIDDM even in the absence of any susceptibility to MODY.

In screening for the location of loci linked to MODY in the RW family, we examined genes involved in carbohydrate and lipid metabolism as well as random DNA polymorphisms. Markers were selected primarily on the basis of heterozygosity of >30% and a genetic distance between markers of ≈20 centimorgans. We analyzed 79 DNA markers including at least one marker on each autosome except chromosome 18. None of these markers showed linkage to MODY and in most instances it was possible to exclude MODY from the vicinity of the marker with a logarithm of odds (lod score) of −2 at a recombination fraction of >0.1. We estimate that we excluded ≈40% of the genome as the site of the MODY gene including ≈25%, ≈75%, and ≈50% of chromosomes 1, 4, and 19, respectively. Candidate genes excluded as the cause of NIDDM in the RW pedigree include insulin; glucagon; insulin receptor; glucose transporter isoforms GLUT1, GLUT2, GLUT3, and GLUT4; hexokinase I; low density lipoprotein receptor; apolipoprotein A-I–C-III–A-IV and C-I–C-II–E complexes; apolipoproteins A-II and B; hepatic and lipoprotein lipases; amylase; and major histocompatibility complex (11–14).

Linkage analyses between the subset of individuals described above and an AluVpA in *ADA* (15) (Fig. 2) on the long arm of chromosome 20 gave a maximum lod score of 1.90 at a sex-averaged recombination frequency of 0.16 using model 2 (incompletely penetrant). Inspection of the pedigree revealed that there were no obligate recombinants between this marker and MODY in the descendants of II-5, and there was no evidence for linkage in the descendants of II-3 and II-6 for whom we had data. Since NIDDM was present in all generations in descendants of II-2 and II-5 and absent among descendants of II-3 in generations IV and V and descendants of II-6 in generations III and IV, we considered the possibility that the NIDDM segregating among the descendants of II-3 and II-6 was not MODY but rather another form of NIDDM. To incorporate this possibility into our analysis, we refined the criteria for the diagnosis of MODY. Only individuals in whom NIDDM was diagnosed at age ≤25 years were assumed to have MODY (Fig. 1); the disease status of all other individuals with diabetes mellitus or impaired glucose tolerance was coded as diagnosis unknown. This age cutoff was chosen because when individuals were tested throughout adolescence, diagnosis of MODY was always made before they were 25 years old (5, 6). For some older family members, the age of diagnosis represented the age at their first biochemical examination; the age at onset in these individuals may have occurred before 25 years but was not detected because they had not been tested. Thus, diabetic subjects diagnosed after 25 years may have MODY or another form of NIDDM. For purposes of analysis, individuals with an age at diagnosis of >25 years are put in a formal category called "diagnosis unknown." The linkage analysis program (LIPED) assigns them an appropriate probability of having MODY, based on their parents, offspring, and the penetrance assumed in the analysis. For some diabetics with diabetic parents, MODY offspring, and MODY siblings, this probability approaches 100%; this is a conclusion from the linkage analysis, not an assumption.

DNA was also obtained for other members of the pedigree. Using the more stringent diagnostic criteria and typing the additional members of the pedigree, we found that the maximum lod score for linkage between MODY and *ADA*

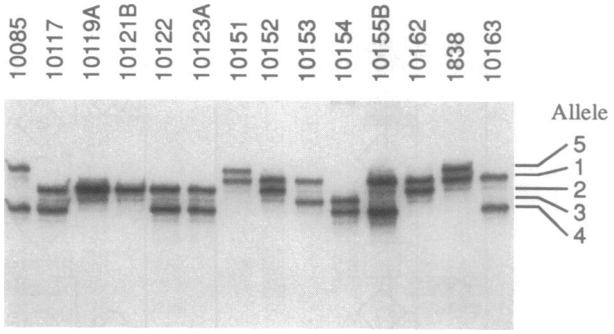

FIG. 2. Analysis of *ADA* AluVpA DNA polymorphism. Oligonucleotides OL3 and OL4 were used to amplify this polymorphism as described in ref. 15. An autoradiograph of the polymerase chain reaction products is shown. The numbers above each lane identify individuals in the RW pedigree (NIGMS Human Genetic Mutant Cell Repository and Fig. 1). The designations for the alleles at this polymorphic site are shown on the right.

was 5.25 at a recombination fraction of $\theta = 0.00$ (0–0.11; 1 lod unit confidence interval) using the highly-penetrant model (Table 1). The *ADA* AluVpA allele 4 segregates with MODY; there are no obligate recombinants. However, one individual (no. 10018, indicated by * in Fig. 1) has inherited the *ADA* AluVpA allele that cosegregates with MODY but had normal glucose tolerance when last tested at 17 years of age; thus, he is not through the age of risk. The linkage data strongly suggest that all the descendants of II-5 with NIDDM have inherited the MODY gene, including those individuals who were first diagnosed as having NIDDM after 25 years of age. Similarly, the descendants of II-2 with NIDDM likely have MODY, with the possible exception of individual 10165, who although she has diabetes has six children, none of whom is known to be affected. The results also suggest that II-3 and his descendants who have NIDDM as well as II-6 do not have MODY but have another form of NIDDM.

The results are robust with respect to assumptions concerning the allele frequency for MODY ($Z_{max} = 5.23$ at $\theta = 0.00$ for $q = 0.01$) or the presence of phenocopies ($Z_{max} = 5.24$ at $\theta = 0.00$ for male and female normal homozygote penetrances = 0.001, corresponding to 80% of affected individuals in the general population being phenocopies). In addition, the uncertainty resulting from reclassifying those subjects with NIDDM diagnosed after the age of 25 years as unknown does not greatly reduce the evidence for linkage. For example, if the age cutoff for MODY is set at 32 years, all members of generation III who have offspring with MODY would then meet the criteria for MODY. In linkage analyses performed under this assumption, and with individuals in generation III who were diagnosed with NIDDM after age 32 years classified as unaffected (with MODY), and individuals with NIDDM in generations I and II considered as unknown, the lod score only increases from 5.25 to 5.63. Even when we used less rigorous diagnostic criteria for defining MODY (i.e., all NIDDM subjects in this pedigree have MODY), there was possible evidence for loose linkage between MODY and *ADA*; the maximum lod score was 2.39 at $\theta = 0.21$ using the incompletely penetrant model (Table 1). However, using the less rigorous diagnostic criteria and analyzing only II-5 and his descendants, the maximum lod score was 4.13 at $\theta = 0.00$.

The analyses described above provided strong evidence for localization of a MODY gene to chromosome 20q and linkage with *ADA*. However, there was still some uncertainty about the proximity of MODY to *ADA*. To address this issue, we examined the segregation of two markers flanking *ADA*, GPR112 (M. Nishi, Y. Yamada, S. Seino, and G.I.B., unpublished data) and D20S27 (16). The results presented in Table 1 (last three lines) strongly suggest that *ADA* is located between GPR112 and D20S27, with recombination fractions of 0.14 and 0.28, respectively. If MODY were only loosely linked to *ADA*, we might expect MODY to be more closely linked to one of these flanking markers than to *ADA*. The results in Table 1 show that this is not the case. Under comparable assumptions (model 2, all NIDDM in pedigree considered to be MODY), MODY shows recombination fractions of 0.19 and 0.29 with GPR112 and D20S27, respectively; these values are similar to the recombination fractions between these markers and *ADA*.

These results are consistent with close linkage between *ADA* and MODY. They also suggest that the NIDDM in the RW pedigree is heterogeneous and that some diabetic members of this family, especially among the offspring of individual II-3, have a non-MODY form of NIDDM.

Although MODY and IDDM have different etiologies, it is not unreasonable to assume that MODY susceptibility genes may in some instances contribute to the development of IDDM, especially in a pedigree such as the one described here in which individuals with MODY have a decreased insulin secretory response and 32% are insulin requiring. There are two subjects with IDDM in the RW pedigree. One patient with IDDM (GM1838; *ADA* AluVpA genotype is 15) is a descendant of II-4 and is in a branch of the family in which MODY is not segregating. The second IDDM subject, individual 1410 (indicated by # in Fig. 1), has a father (individual 1246) and two siblings (individuals 7974 and 10211) with MODY. However, she did not inherit the *ADA* allele that cosegregates with MODY. Thus, in neither of these individuals was the development of IDDM influenced by the MODY gene.

The linkage data presented in this report establish that the gene responsible for the development of MODY in the RW family is located on the long arm of chromosome 20. Studies of the segregation of this locus in other MODY families as well as in families with late age of onset forms of NIDDM will indicate its relevance to our understanding of NIDDM. The identity of the MODY gene is unknown. It is unlikely to be *ADA* since diabetes mellitus is not a feature of adenosine deaminase deficiency in either humans or mice, and the list

Table 1. Pairwise lod scores for linkage between MODY and DNA polymorphisms on chromosome 20

Marker	MODY diagnosis	Model	Lod score at recombination fraction (θ) of								Z_{max}	at θ
			0.00	0.001	0.01	0.05	0.10	0.20	0.30	0.40		
ADA	≤25 yr	1	5.25	5.25	5.18	4.81	4.35	3.33	2.22	1.04	5.25	0.00
ADA	≤25 yr	2	4.85	4.84	4.77	4.44	4.00	3.07	2.07	0.98	4.85	0.00
ADA	All	1	−∞	−18.69	−10.13	−3.25	−0.45	1.61	1.91	1.21	2.03	0.27
ADA	All	2	−4.35	−3.74	−1.94	0.67	1.75	2.33	2.02	1.12	2.39	0.21
GPR112	≤25 yr	1	−5.46	−4.49	−2.14	0.03	0.94	1.46	1.29	0.77	1.46	0.22
GPR112	≤25 yr	2	−0.74	−0.66	0.54	1.76	2.10	2.00	1.49	0.77	2.14	0.13
GPR112	All	1	−∞	−10.36	−6.09	−2.50	−0.74	0.80	1.12	0.78	1.12	0.30
GPR112	All	2	−4.85	−4.62	−2.02	0.50	1.36	1.71	1.40	0.78	1.71	0.19
D20S27	≤25 yr	1	−13.81	−8.83	−4.89	−1.72	−0.37	0.57	0.67	0.41	0.69	0.27
D20S27	≤25 yr	2	−9.51	−6.13	−3.56	−1.02	−0.03	0.65	0.70	0.44	0.73	0.26
D20S27	All	1	−∞	−30.93	−18.29	−8.31	−4.15	−0.73	0.40	0.43	0.53	0.35
D20S27	All	2	−11.64	−8.47	−5.65	−2.25	−0.69	0.60	0.87	0.54	0.87	0.29
ADA/GPR112			−∞	−7.85	−1.76	2.26	3.37	3.24	2.17	1.06	3.53	0.14
ADA/D20S27			−∞	−54.62	−27.78	−9.73	−2.92	1.88	2.65	1.61	2.68	0.28
GPR112/D20S27			−∞	−38.78	−22.55	−10.99	−6.20	−2.08	−0.47	−0.02		

The pairwise lod scores between MODY and chromosome 20 DNA polymorphisms are shown, as are the scores between *ADA* and GPR112 or D20S27, and GPR112 and D20S27. All lod scores were calculated by using sex-averaged recombination frequencies. GPR112 is the gene for a member of the G protein-coupled receptor superfamily having a dinucleotide-repeat polymorphism in its 5′-flanking region (M. Nishi, Y. Yamada, S. Seino, and G.I.B., unpublished data). The scores obtained using the two different diagnostic criteria for the definition of MODY (see text) are shown, as are the results for the highly penetrant and incompletely penetrant models for MODY, models 1 and 2, respectively.

of genes that have been mapped to chromosome 20 (17) provides no clues as to its identity. However, knowledge of the location of the MODY gene will facilitate its isolation and identification of the mutation that impairs its function.

This report indicates the feasibility of using linkage to identify NIDDM-susceptibility genes. As clinical data indicate that the MODY phenotype is heterogeneous (5, 6), similar studies of other MODY families may lead to the identification of additional diabetes-susceptibility genes.

This study was supported by the Howard Hughes Medical Institute, a gift from Sandoz Research Institute, and grants from the National Institutes of Health, Michigan Diabetes Research and Training Center, and the Philadelphia chapter of the American Diabetes Association.

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